RESEARCH PAPER

The mixed culture of microalgae *Chlorella pyrenoidosa* and yeast *Yarrowia lipolytica* for microbial biomass production

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Abstract
Microbial biomass which mostly generated from the microbial processes of bacteria, yeasts, and microalgae is an important resource. Recent concerns in microbial biomass production field, especially microbial lipid production for biofuel, have been focused towards the mixed culture of microalgae and yeast. To more comprehensive understanding of the mixed culture for microbial biomass, mono *Chlorella pyrenoidosa*, mono *Yarrowia lipolytica* and the mixed culture were investigated in the present work. Results showed that the mixed culture achieved significantly faster cell propagation of microalga and yeast, smaller individual cell size of yeast and higher relative chlorophyll content of microalga. The mixed culture facilitated the assimilation of carbon and nitrogen and drove the carbon flow to carbohydrate. Besides higher lipid yield (0.77 g/L), higher yields of carbohydrates (1.82 g/L), protein (1.99 g/L) and heating value (114.64 kJ/L) indicated the microbial biomass harvested from the mixed culture have more potential utilization in renewable energy, feedstuff, and chemical industry.

Keywords *Chlorella pyrenoidosa* · *Yarrowia lipolytica* · Mixed culture · Cell morphology · Microbial biomass

Introduction

Microbial biomass which is mostly generated from the microbial processes of bacteria, yeasts and microalgae contains a large amount of valuable components for food, feed, fertilizer and biofuels production. Recently, microalgae have attracted wider attention for its valuable application on fuel, food and pharmaceuticals products. *Chlorella* genus is a widely available microalga and has many commercial applications including food supplements, feed additives, biofuel feedstocks, etc. [1]. Yeast is considered as potential microbial resources for its relatively shorter doubling time, less affected by the environmental stress compared to other microbes. *Yarrowia lipolytica* is a typical species of yeast which is classified as GRAS (generally recognized as safe) for human [2]. Furthermore, *Y. lipolytica* displays a broad carbon spectrum (glycerol, glucose and hydrophobic substrates etc.) and is known as an extracellular protein-secreting species makes it plays an important role in the process of biosynthesis, biodegradation and biotransformation [3–5].

The mixed culture of microorganism has been used in many bioprocesses including waste degradation, remediation and biomass generation for bioactive compounds production [6]. The recent energy crisis has triggered significant attention on the microbial lipids which are used as feedstock for biodiesel production. Recent concerns in the microbial lipid production field for biofuel production have been focused towards the mixed culture of microalgae and yeast. A number of microalgae and yeast species in various combinations have been studied and the enhanced lipid production in the mixed culture was confirmed [7–10]. For example, 40–50%

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increases of total lipid were obtained in the mixed culture of *Rhodotorula glutinis* and *Scenedesmus obliquus* in a 5-L photobioreactor compared to mono-cultures alone [11]. In addition to lipid, microbial biomass also contains large amounts of carbohydrates and proteins. Carbohydrates are feedstock for alternative biofuels (bioethanol) and value-added chemicals via catalytic pyrolysis [12]. Single-cell proteins (SCPs) are important protein supplement in human foods or animal feeds. Hence, study of other associated main components (carbohydrates and protein) assimilation in cells is essential for better understanding of the advantages of the mixed culture. In research of synergistic effects between microalga and yeast in the mixed culture, O_2/CO_2 balance, substrate exchange, dissolved oxygen, pH adjustment, and the release of trace elements have been expounded to clarify the symbiotic relationship which may exist between yeast and microalga, and it is beneficial in terms of increasing biomass and lipid yield [10, 11, 13]. However, the effects of individual microalgae and yeast cell in the mixed culture system have not been studied well and the related information is not available.

In this study, to expand the perspective of the mixed culture for microbial biomass production and discuss the potential biomass utilization, the nitrogen and organic carbon removal, biomass growth, cell characteristics of *C. pyrenoidosa* and *Y. lipolytica*, biochemical profile of biomass including carbohydrate and protein content, and the higher heating value (HHV) of biomass were compared among three systems (mono *C. pyrenoidosa*, mono *Y. lipolytica* and mixed culture of *C. pyrenoidosa* and *Y. lipolytica*). This research will provide wider insight for the mixed culture of microalga and yeast, and promote the establishment of a full-component utilization scheme for microbial biomass.

Materials and methods

Strains and culture medium

The microalga *C. pyrenoidosa* 15–2070 was obtained from Carolina Biological Supply Company, USA. The yeast *Y. lipolytica* GIM2.197 was obtained from Guangdong Microbial Culture Collection Center, China. The seed media of microalga and yeast were BG11 and YPD, respectively. The medium used for the mono- and mixed cultures was described as follows: 1-L medium containing glycerol 7 g, yeast extract 2 g, NH_4HCO_3 0.12 g, citric acid 6.0 mg, ferric ammonium citrate 6.0 mg, EDTA 1.0 mg, $NaNO_3$ 1.5 g, $K_2HPO_4 \cdot 2H_2O$ 0.051 g, $MgSO_4 \cdot 7H_2O$ 0.075 g, $CaCl_2$ 0.024 g, Na_2CO_3 0.02 g, A5 trace mineral solution 1.0 mL. The composition of A5 was: H_3BO_4 2.86 g/L, $MnCl_2 \cdot 4H_2O$ 1.81 g/L, $ZnSO_4 \cdot 7H_2O$ 0.222 g/L, $Na_2MoO_4 \cdot 2H_2O$ 0.391 g/L, $CuSO_4 \cdot 5H_2O$ 0.079 g/L, $Co(NO_3)_2 \cdot 6H_2O$

0.049 g/L. All media were sterilized at 121 °C for 20 min before use, NH_4HCO_3 was added into the sterilized medium after filtration by a 0.2-μm filter unit (Millex-GP, Millipore).

Seed culture and experimental set-up

The yeast *Y. lipolytica* was cultivated in YPD medium at 28 °C and 150 rpm for 24 h. The microalga *C. pyrenoidosa* was cultivated at 28 °C and 150 rpm for 4 days under illumination at 45 ± 3 μ mol photons/m²/s with 2% CO_2 supplemented from the bottom of the flasks. For preparing the high cell density inoculums, the seed cultures were centrifuged at 4000 rpm for 5 min at 25 ± 1 °C, and the precipitate was diluted suitably using sterilized water.

The mono- and mixed cultures were performed in shaker using 250-mL flasks with 75-mL working volume and cultivated at 28 ± 1 °C, and 170 rpm with the light supply of 45 ± 3 μmol photons/m²/s. The initial cell counts of microalga and yeast after inoculation were 7.5×10^6 and 2.0×10^5 cells/mL, respectively. Flasks containing three kinds of culture were taken out from shaker at 24 h, 48 h, 72 h, 120 h and 168 h and the samples were used for analyses. All the experiments were carried out in triplicate.

Analytical methods

Measurement of biomass and pH

Total 3 mL of culture was collected and centrifuged (Centrifuge 5804 R, Eppendorf AG, Hamburg, Germany) at 6000 rpm for 4 min. Cells were washed twice by distilled water and dried at 60 °C for biomass dry weight determination. A pH meter (Five Easy Plus, Mettler-Toledo, Australia) was used to determine the pH of the culture.

Single-cell analysis by flow cytometry

1 mL of culture was centrifuged at 6000 rpm for 5 min. Cells were washed with distilled water and diluted to 1×10^6 cells/mL for analysis of cell counting, individual cell size and fluorescent intensity of chlorophyll by flow cytometry (Beckman CytoFLEX, Germany). Cell population of microalga and yeast in the mixed culture was distinguished via algal chlorophyll auto-fluorescence. The data were analyzed and performed using FlowJo Version 8 software (TreeStar, Ashland, CA, USA).

The mean specific growth rate (μ_{Mean}) was calculated using cell concentration data from flow cytometry using Eq. 1.

$$\mu_{Mean} = \ln \left(C_{168} - C_0 \right) / \left(t_{168} - t_0 \right), \tag{1}$$

where C_{168} and C_0 are the cells concentration at time t_{168} and t_0, respectively. The μ_{Mean} is expressed in d⁻¹. Individual

cell size can be described by forward scatter (FSC) measurement, specifically the integral ("area") of a FSC pulse (FSC-A) [14]. The change of cell size was calculated using FSC-A value from flow cytometry (Eq. 2).

$$\text{Change of cell size} = \frac{F_{tn} - F_{t0}}{F_{t0}} \times 100\%, \tag{2}$$

where F_{tn} is the FSC-A value at 24 h, 48 h, 72 h, 120 h and 168 h, respectively; F_{t0} is the FSC-A value at 0 h.

Red fluorescence (670 nm) is linearly correlated with the chlorophyll content of living microalga cells [15]. The mean of integral fluorescent signal of PC5.5 channel (PC5.5-A) which with 488 nm Laser and 690/50 Filter was used to characterize relative chlorophyll content of microalgae in the mono- and mixed cultures.

Medium composition detection

Total nitrogen (TN), and ammonium (NH₃-N) were determined using a Hach DR2700 Spectrophotometer (Hach Co., USA) and Hach reagents (CAT No.2714100, and 2606945, respectively) following the manufacturer's procedure. NO_3^- was determined using a Metrohm ion chromatograph (883 Compact IC Pro, Switzerland) equipped with a Metrosep A Supp4-250 analytical column. The total organic carbon (TOC) was determined using an elemental analyzer (Vario TOC, Elementar, Hanau, Germany). A modified colorimetric method based upon cupric–glycerol complex was used to determine the glycerol concentration [16]. Briefly, 0.3 mL of 15% copper sulfate solution and 3.5 mL of 5% sodium hydroxide solution were reacted to generate copper hydroxide suspension. Then, 0.4 mL of either glycerol standard solution or sample supernatant was added into abovementioned copper hydroxide suspension. After vigorous vortex for 1 min, the well-mixed solutions were centrifuged at 5,000 rpm for 3 min, and clear supernatants were collected and measured for absorbance at 630 nm.

Biomass composition and HHV analysis

Carbohydrates content was analyzed by phenol–sulfuric acid method [17]. Total lipid content in the dry biomass was determined by solvent extraction [18]. About 5 mg of freeze-dried biomass was used for elemental analysis using vario EL cube (Elementar, Germany). Crude protein content was determined and expressed as N% × 6.25 [19]. HHV was calculated using well-established correlations (Eq. 3) given by [20], based on the elemental composition.

$$HHV = 3.55C^2 - 232C - 2230H + 51.2C \times H + 131N + 20600, \tag{3}$$

where C, N, and H represent carbon, nitrogen, and hydrogen contents of material, respectively, expressed in mass percentages.

Statistical analysis

All the experiments were conducted in triplicate and the data were presented with the mean and standard deviation unless the specifically states otherwise. Statistical analyses were performed using the Data Analysis Tool Pak available on Microsoft Excel. Two-factor analysis of variance without replication analysis was used to estimate the statistical significant differences.

Results and discussion

Removal of nitrogen and organic carbon in mono- and mixed cultures

Nitrogen is an essential nutrient and required for the growth of all organisms. In nature, most of the microorganisms have developed their own mechanisms for uptake and assimilation of mineral either forms of nitrogen (such as ammonium and nitrate) or organic forms of nitrogen [21]. To have a comprehensive knowledge of the removal and uptake of different forms of nitrogen in the mono- and mixed cultures, TN (495.00 mg/L) which was mainly composed of NH₃–N (35.17 mg/L), NO₃–N (278.53 mg/L) and organic nitrogen was introduced in the culture system. It can be noticed that the mixed culture enhanced the removal of nitrogen (Fig. 1). The TN removal rate of the mixed culture (66.33%) was higher than that of mono-culture of yeast (31.99%) and microalga (17.85%) at the end of cultivation (Fig. 1a). The result was consistent with previous studies, which reported that the removal efficiencies for TN for the mixed culture (51.18%) were higher than those for the mono-culture of microalgae (29.28%) and yeast (46.31%) [9]. Although 100% NH₃–N removal was achieved in the mixed culture and the mono yeast culture during the first 24 h, there was a sharp NH₃–N concentration increase after 72 h in the mono yeast culture. The mono microalgae culture exhibited continuous removal abilities of NH₃–N and 68.15% removal rate was reached at the end of cultivation (Fig. 1b). Continuous removal of NO₃–N was observed in the mixed culture and 42.07% removal rate was achieved at the end of culture, while no removal was recorded in the mono microalgae culture and the mono yeast culture (Fig. 1c).

Biological uptake and accumulation are considered to be a major way of nitrogen removal as nitrogen is an essential element for various biological substance syntheses (e.g., protein, nucleic acid, and phospholipid) [22]. In addition to biological removal, ammonia stripping is another important

Fig. 1 Time courses of the concentrations of TN (**a**), NH₃–N (**b**) and NO₃⁻–N (**c**) in the mono- and the mixed cultures
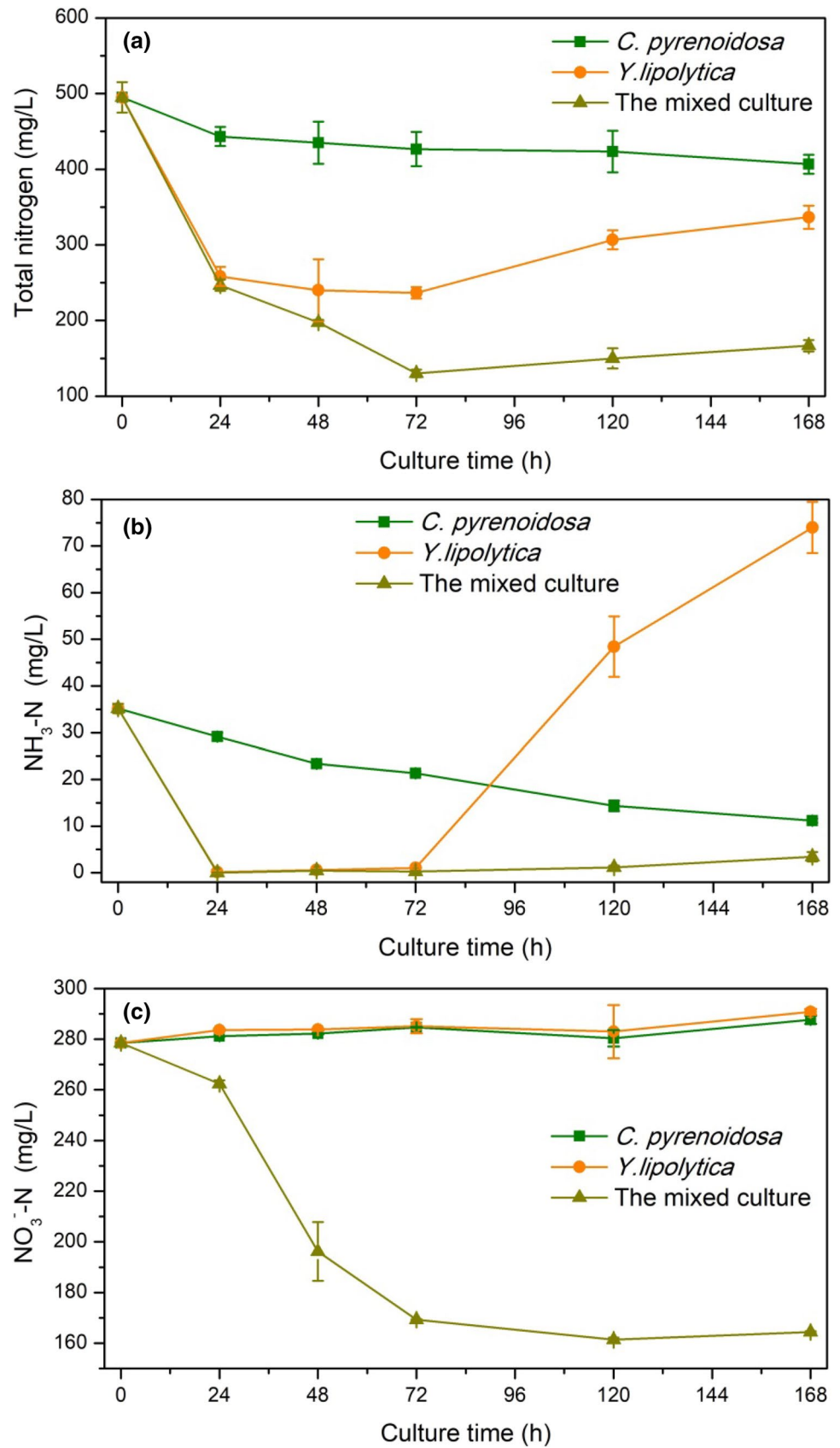
way of nitrogen removal [23]. Ammonium ion–ammonia equilibrium in aqueous phase is governed by pH and temperature, and free ammonia at pH 9.25 can be stripped out of solution by aeration [24]. Since aeration and temperature in our experiment were both constant, the differences of ammonia stripping among the three systems were attributed to pH variation. Apart from the later stage of the mixed culture, pH of three culture systems was below 9.25 (Figure S1). Obviously, nitrogen removal in this work relies mainly on biological uptake and accumulation and the rapid growth of yeast in the mono yeast culture and the mixed culture contributed to removal of NH_3–N in the early stage. It was reported that ammonia release is a mechanism of protection from yeast cell death under limited nutrient conditions [25]. This may be the reason of the NH_3–N concentration increase in mono yeast culture after 72 h; at this time point, the total organic carbon almost exhausted (Fig. 2a). NH_3–N concentration increase was not been observed at the later stage of the mixed culture may be due to the released NH_3–N was stripped out of solution which was caused by high pH.

Unlike *Chlorella,* which can utilize ammonia and nitrate nitrogen for growth, *Y. lipolytica* cannot utilize NO_3–N for growth [26]. No removal of NO_3–N in the mono yeast culture was logical. For microalgae, ammonium is more preferred than nitrate since a redox reaction is not involved [23]. It is widely documented that nitrate consumption does not occur until the ammonium is almost completely consumed [27]. Hence, the presence of ammonia nitrogen stopped NO_3–N assimilation completely in the mono microalga culture.

Glycerol can be used as a good carbon source by *Y. lipolytica*. When both glycerol and glucose were contained in the

Fig. 2 Time courses of the concentrations of TOC (**a**) and glycerol (**b**) in the mono- and the mixed cultures
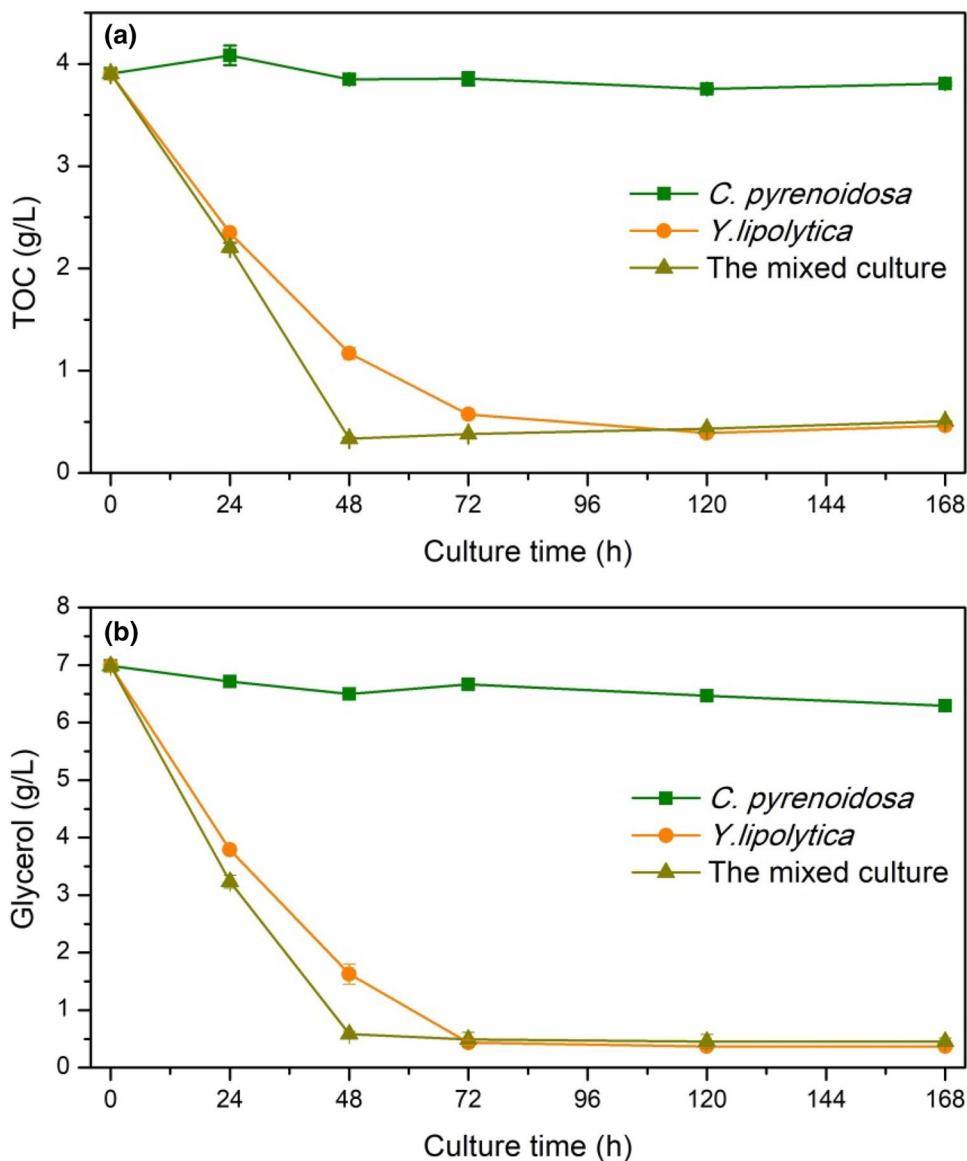
culture medium, glycerol was used firstly by yeast [4]. In this study, glycerol (initial concentration 7 g/L) was introduced into culture system as a carbon source. The profile of TOC was basically consistent with glycerol (Fig. 2). The mixed culture displayed obvious advantage of glycerol uptake in the early stage of culture. However, with the culture proceeding, the advantage disappeared. The mono yeast culture and the mixed culture exhibited remarkable uptake rate (94.72% and 93.48%), which was higher than that of the mono microalga culture (just 9.94%). Although *C. pyrenoidosa* could grew in mixotrophic condition in the presence of glycerol [28], its glycerol utilization capacity obviously inferior to *Y. lipolytica*.

Biomass growth and cell characteristics of *C. pyrenoidosa* and *Y. lipolytica*

As shown in Fig. 3a, the mixed culture displayed significant advantage ($p < 0.01$) in biomass production than mono microalga and mono yeast culture during the whole process. The highest biomass concentration (5.77 g/L) and mean biomass productivity (0.76 g/L/d) were achieved by mixed culture (Table 1). This result is consistent with the previous studies which reported mixed culture of microalgae and yeast can enhance the biomass production [6, 13, 23]. The highest biomass obtained in this study was higher than that from the mixed culture of *R. glutinis* and *Chlorella vulgaris* (4.63 g/L) [6] and that from the mixed culture of *Y. lipolytica* and *C. vulgaris* (1.62 g/L) [23]. It is widely documented that there was symbiotic relationship including O_2/CO_2 exchange, pH adjustment and substance exchange between microalga and yeast in the mixed culture, further providing

Fig. 3 Biomass concentration (**a**) and the respective growth curves of microalga and yeast cell (**b**) in the mono- and the mixed cultures. ** represent the significant difference between the mixed culture and mono-cultures ($p < 0.01$), asterisks and hashes represent the significant difference of cell concentration between the mixed- and mono-cultures of corresponding microalgae and yeast ($p < 0.05$), respectively
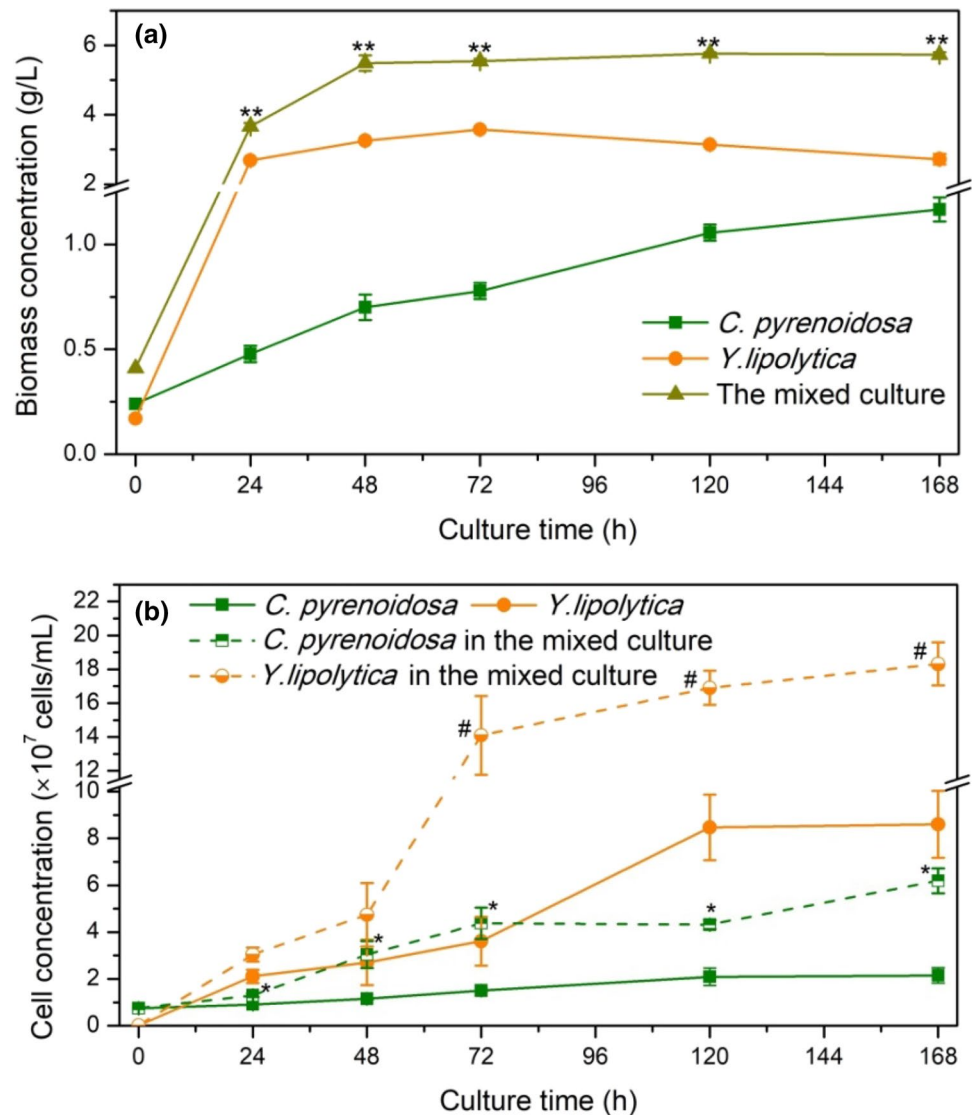
Table 1 Kinetic parameters of biomass production and cell growth in the mono- and mixed cultures

	X_{max} (g/L)	Biomass productivity (g/L/d)	μ_{Mean} (d^{-1})
C. pyrenoidosa	1.17 ± 0.06	0.13 ± 0.01	0.15 ± 0.02
Y. lipolytica	3.58 ± 0.04	0.36 ± 0.02	0.88 ± 0.02
The mixed culture	5.77 ± 0.03	0.76 ± 0.01	0.30 ± 0.02[a]
			0.99 ± 0.01[b]

X_{max}: the maximal biomass concentration reached during the cultures at 168, 72 and 120 h for the mono- culture of *C. pyrenoidosa*, *Y. lipolytica* and the mixed culture, respectively
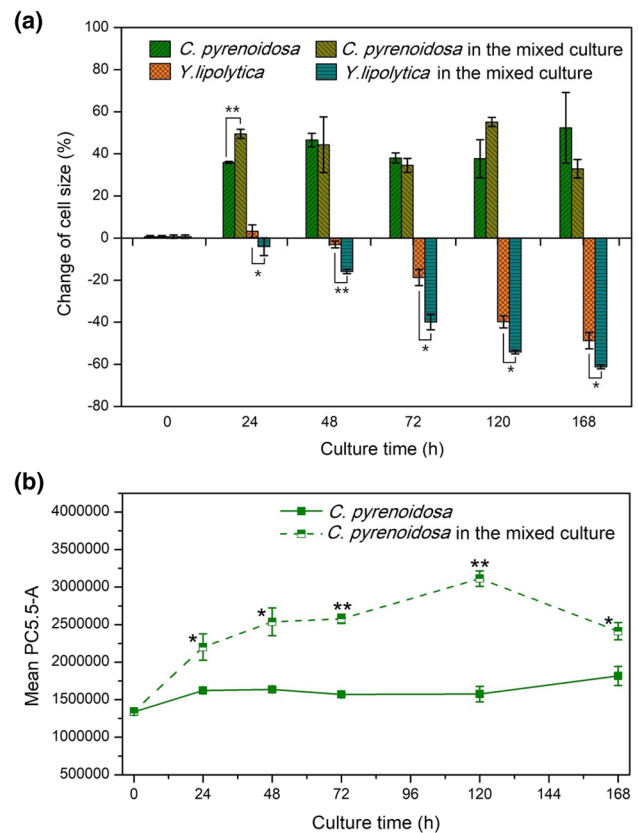
[a]Significant difference between the mixed- and mono-culture of microalgae at level ($p < 0.05$)

[b]Significant difference between the mixed- and mono-culture of yeast at level ($p < 0.01$)

higher biomass production compared with the mono-culture [6, 13].

In this study, flow cytometry (FCM) was used for cell counting. The Chlorophyll fluorescence of microalgae can enable to count the microalgae and yeast separately by setting an FCM gate [29]. As shown in Fig. 3b, cell concentration of microalgae and yeast in the mixed culture was significantly higher than that in the corresponding mono-culture. Specifically, the mean specific growth rate of microalga (0.30 day^{-1}) and yeast (0.99 day^{-1}) in the mixed culture was significantly higher than that of the mono-culture of microalga (0.15 day^{-1}) and yeast (0.88 day^{-1}), respectively (Table 1). This indicated that synergistic effect may exist in the mixed culture which was beneficial to cell propagation of microalga and yeast. Interestingly, the maximal biomass in the mixed culture and the mono-culture of yeast was basically reached before 48 h, while yeast cell concentration sharply increased after 48 h. In order to comprehensive understanding the enhanced biomass production in the mixed culture, individual cell sizes of microalgae and yeast, relative Chlorophyll content of microalga cell were investigated.

As shown in Fig. 4a, the individual cell size of yeast was becoming smaller along with the culture proceeding and it was significantly smaller in the mixed culture. The cell growth is actually including cell division (reproduction) and cell development which related to cell size. Budded *Y. lipolytica* cells divide asymmetrically into larger parents and smaller daughters, and need enough resources to maintain average size over the generations [30]. During nitrogen starvation, cells of the yeast *Saccharomyces cerevisiae* increased threefold in number without net biomass growth [31]. Hence, smaller average individual cell size of yeast cell along with the culture proceeding may be caused by the gradual nutrient starvation. Furthermore, the increase of dissolved oxygen concentration can cause the reduction of yeast

Fig. 4 Individual cell size of microalga and yeast (**a**) and the relative chlorophyll fluorescent intensity of microalga cell (**b**) in the mono- and the mixed cultures. Asterisks represent the significant difference between the mono- and mixed cultures at different levels (*$p < 0.05$ and **$p < 0.01$)

cell size [32]. Hence, higher O_2 concentration caused by the photosynthesis of microalgae in the mixed culture could lead to smaller individual cell size of yeast in the mixed culture.

In terms of *C. pyrenoidosa*, the individual cell size after inoculation both in the mono *C. pyrenoidosa* culture and mixed culture has an increase in 30–50%. Microscopic photographs also show the same results (Figure S2). Comparing with BG11 which was used as seed media, media used for the mono- and mixed cultures provide more adequate nutrition. This may increase the individual cell size of *C. pyrenoidosa* after inoculation as the average individual cell size depends on surrounding environment [33]. When we focused on the comparison of the mono-culture and mixed culture, microalga is with significantly bigger individual cell size than mono *C. pyrenoidosa* culture at 24 h. It has been reported that microalgae with a bigger individual cell size when growth at high CO_2 [34]. Hence, higher CO_2 concentration caused by the aerobic respiration of yeast in the mixed culture might cause this result. After 24 h, the weaken growth of yeast and the continuous photosynthetic autotrophic growth of algae could result in a decrease of CO_2

concentration [13], which caused the change of microalga cell size in the mixed culture not obvious comparing with mono *C. pyrenoidosa* culture. It was reported that individual cell size of algae was also affected by light intensity, being bigger at low light intensity [35]. Hence, the attenuation of light intensity caused by the increased yeast cell concentration might contribute to the large cell size of microalgae in the mixed culture. Furthermore, light intensity is also one of the factors having effect on the chlorophyll content. It was reported that the increase of total chlorophyll content is one way of physiologically adapting to reduced irradiance in terrestrial plants and algae [36]. Hence, the attenuation of light intensity in the mixed culture might result in the increase of the chlorophyll content per microalgae cell (Fig. 4b).

Biomass biochemical profile of mono- and mixed cultures

In many microorganisms, multiple forms of storage carbon products can be found, such as carbohydrates and lipids. As biosynthesis of the multiple forms of storage compounds may require the same carbon precursors and reducing power, thus understanding the change rule of carbon storage is the key issue of biomass enhancement in the mixed culture (Fig. 5a, b). Total carbohydrates content of the mixed culture is higher than those of the mono microalga culture and the mono yeast culture. Total carbohydrate contents were uptrend in the mixed culture (17.18–31.72%), the mono microalga culture (16.18–19.2%) and the mono yeast culture (14.14–24.68%). Contrary to carbohydrate contents, the total lipid contents after 72 h were downtrend in the mixed culture and the mono-culture. Total lipid content of the mixed culture was higher than that of the mono yeast culture and lower than that of the mono microalga culture. It was concluded that assimilated carbon was mainly flow to the carbohydrates, and this effect has been strengthened in the mixed culture. Lipids and carbohydrates likely compete with each other for carbon units, and accumulation of storage lipids either occurs in parallel or lags behind the accumulation of storage carbohydrates [37]. Carbohydrates are most likely preferred as reserve metabolites under conditions of short-term nutritional stress, as observed in plants [38]. The biochemical analyses showed that the crude protein content in the mono microalga fluctuates from 50.28 to 54.58%, while there was downtrend along with the culture proceeding in the mono yeast (48.67–29.13%) and the mixed culture (44.06–34.65%) (Fig. 5c). During nitrogen starvation, little protein was accumulated [31]. Hence, the deplete of the available nitrogen in the medium may be the main reason of the decrease in the protein content of the cells in the mono yeast culture and the mixed culture. In addition, the ammonia release at the later stage of culture further aggravated the decrease of protein content in the mono yeast culture [25].

The crude protein content of the mixed culture was lower than that of mono-cultures before 48 h, and then higher than that of the mono yeast culture and lower than that of the mono microalga culture. The profile of HHV, which is a major indicator of biomass quality in energy properties, is given in Fig. 5d. The HHV value of the mono microalga culture was uptrend and the mono yeast culture was downtrend, while the mixed culture displayed uptrend till 72 h and then decreased. HHV value of the mixed culture was higher than that of the mono microalga culture and mono yeast culture till 72 h, and then higher than that of the mono-culture of yeast and lower than that of microalga.

Yield is a key factor for whether one system is suitable for practical and commercial applications. As shown in Table 2, the mixed culture achieved higher yield of protein, carbohydrates, lipid and calorific value. The lipid yield of the mixed culture (0.77 g/L) which was 3.85 times of the mono yeast culture and 4.53 times of mono microalga culture was obtained in the current study. Similar result was also detected in the mixed culture of *C.pyrenoidosa* and *R. glutinis*, in which the maximum lipid productivity in the mixed culture was significantly higher than that of mono microalgae and yeast cultures, measuring 134.05 and 247.94%, respectively [39]. Furthermore, the protein yield of the mixed culture (1.99 g/L) which was 2.52 times of the mono yeast culture and 3.21 times of mono microalga culture; the carbohydrates yield of the mixed culture (1.82 g/L) which was 2.72 times of the mono yeast culture and 7.91 times of mono microalga culture; HHV yield of the mixed culture (114.64 kJ/L) which was 2.35 times of the mono yeast culture and 4.70 times of mono microalga culture were also recorded in the current study.

Potential applications expansion

Most of the studies related to the mixed culture of microalgae and yeast focused on enhancement of lipid production [9, 11, 13]. Excepting lipid, carbohydrates and protein contents are very high in microbial biomass. Carbohydrates are feedstock for alternative biofuels (bioethanol) and value-added chemicals via catalytic pyrolysis [12]. Single-cell proteins (SCPs) are important protein supplement in human foods or animal feeds. Hence, comprehensive and reasonable utilization of biomass components seems more reasonable. Furthermore, thermochemical conversion, which usually can utilize the entire biomass feedstock for biofuel production, is considered as a simpler route to produce biofuels [40]. The technology can be a complement to chemical and biochemical methods for the maximal utilization of microbial biomass [40]. The results of this study suggested that the promising advance of the mixed culture of microalgae and yeast should not be confined to lipid production. It can be extended to enhance the protein and carbohydrates production which can
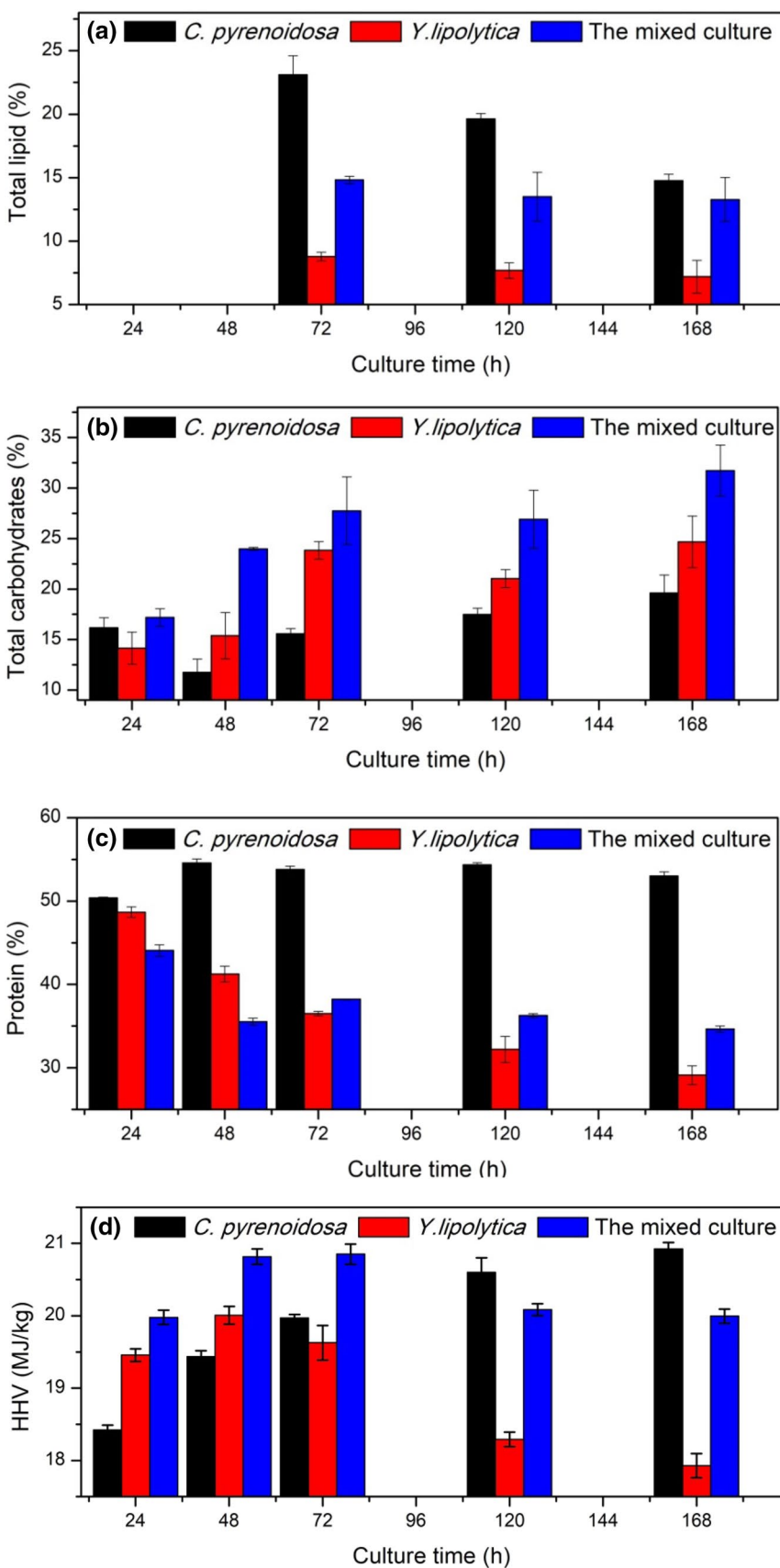
Table 2 Yields of protein, carbohydrate, total lipids and HHV in harvested biomass in the mono- and mixed cultures

	Crude protein (g/L)	Carbohydrates (g/L)	Lipid (g/L)	HHV (kJ/L)
C. pyrenoidosa	0.62 ± 0.02	0.23 ± 0.03	0.17 ± 0.02	24.41 ± 0.11
Y. lipolytica	0.79 ± 0.07	0.67 ± 0.08	0.20 ± 0.05	48.81 ± 0.45
The mixed culture	1.99 ± 0.02	1.82 ± 0.16	0.77 ± 0.11	114.64 ± 0.56

be helpful for food/feeding industry and chemical industry. Furthermore, the mixed culture system has better capacity to provide gaseous, liquid fuel, solid fuel and prepare chemicals if the harvested microbial biomass be utilized by thermochemical conversion.

Conclusion

Besides the higher yield of biomass and lipid, the mixed culture of *C. pyrenoidosa* and *Y. lipolytica* can achieve the higher yields of carbohydrates, protein, and HHV. Aside from benefiting to cell propagation of microalga and yeast, the mixed culture also makes individual cell size of yeast smaller and relative chlorophyll content of microalga higher. Furthermore, the mixed culture could strengthen the carbon flow to carbohydrates biosynthesis. To comprehensively understand the synergistic effect of the mixed culture, the differential transcriptome analysis of microalga and yeast cells in the mono- and mixed cultures is in progress.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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